

Macrophages are a type of white blood cells that play key roles in host defense by recognizing and engulfing foreign and apoptotic bodies. To accomplish this task, they rely on complex molecular interactions involving both lipids and proteins. Previous studies have shown that surface exposure of phosphatidylserine by apoptotic cells is required for their successful clearance, suggesting specific lipid-protein interactions at least for the initiation of phagocytosis of apoptotic cells. However, macrophages can engulf foreign and apoptotic bodies that substantially vary in size suggesting that non-specific interactions over a range of length scales may be relevant. The purpose of our study is to investigate the correlation between physical properties of lipid bilayers and their engulfment by macrophages. We modify bilayer properties systematically as a function of phospholipid headgroup composition and by addition of ceramide and cholesterol. We use a combination of scattering and spectroscopic methods to quantify lipid interactions and flow cytometry to measure engulfment rates. This study can help distinguish between the role of lipids and proteins in clearance of apoptotic and foreign particles.

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#### 2658-Pos Board B350

##### Availability of PIP<sub>3</sub> at the Plasma Membrane Regulates Secretion in Pc12 Cells

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The phosphoinositide PIP<sub>2</sub> has long been implicated in secretion; however despite the demonstration of high affinity binding between synaptotagmin and PIP<sub>3</sub>, the role of the latter in secretion has received little attention. We have investigated this question using two complementary approaches; expression of PIP<sub>2</sub> or PIP<sub>3</sub> targeting Pleckstrin Homology (PH) domains, and up or down regulation of the PIP<sub>3</sub> dephosphorylating enzyme PTEN (Phosphatase and TENsin homolog). The PH-domain of GRP1 (General receptor for phosphoinositides), which we show to have high specificity for PIP<sub>3</sub>, is equally as effective at inhibiting secretion as the PH domain of phospholipase C delta 1, which is generally accepted as binding to PIP<sub>2</sub>. Using super-resolution Stochastic Optical Reconstruction Microscopy we have shown that PIP<sub>2</sub> and PIP<sub>3</sub> clusters do not colocalize, and find that PIP<sub>3</sub> clusters, but not PIP<sub>2</sub> clusters, are reduced in number by over expression of PTEN (Phosphatase and TENsin homolog). Analysis of single vesicle rates of secretion with TIRF (Total Internal Reflection Fluorescence) microscopy shows a correlation between secretion rates and cellular PTEN content. These results implicate PIP<sub>3</sub> in the secretory machinery, and may illuminate some of the neurological impacts of PTEN mutations, such as epilepsy and Autism Spectrum Disorders.

#### 2659-Pos Board B351

##### Comparison of Three Algorithms for Detection of Amperometric Spikes Resulting from Quantal Exocytosis

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Electrochemical microelectrodes located immediately adjacent to the cell surface can detect spikes of amperometric current during quantal exocytosis as the transmitter released from a single vesicle is oxidized on the electrode surface. Automated techniques are needed to detect spikes that vary considerably in amplitude and time course. We compared three algorithms for spike detection against the gold standard of manual detection. The most widely used method for spike detection is testing when the current slope (first derivative) exceeds a threshold (DT method). The DT method is sensitive to the selection of both the low-pass filter settings and the threshold. A second method is to scan the data set with one or more prototype spike templates while performing a least-squares fit to find the amplitude and standard error of the fit at each time point. A spike is identified when the ratio of the fit amplitude to the standard error exceeds a threshold (CB method). This method is not as sensitive to filter settings or threshold but is sensitive to the choice of template(s). Finally, we tested a new approach (DLS method) that is similar to the CB method but performs a least-square fit of the sum of two templates consisting of faster and slower exponential decays. Receiver Operator Characteristic plots demonstrate that the DT method fails to detect slower spikes without generating significant false positives. The CB method also fails to detect some spikes manually identified when using up to three templates. On the other hand, the DLS method detected a much wider range of spike types with the use of only two spike templates. We conclude that the DLS method enables detection of a large variety of spikes without excessive computational cost. Supported by NIH R43MH06650.

#### 2660-Pos Board B352

##### NeuroSensor 521: A Fluorescent Sensor for Selective Labeling of Norepinephrine-Containing Vesicles

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We have developed a novel fluorescence-based turn-on molecular sensor (NeuroSensor 521) that selectively binds to primary amines via iminium ion formation. The fluorescence increases > 5-fold upon binding norepinephrine and is accompanied by a red shift in the absorption maximum from 448 to 488 nm. Norepinephrine and dopamine bind with a ~10-fold higher affinity (~10 mM) than other alkyl amines such as glycine, but secondary amines such as epinephrine do not have a measurable binding affinity. We used confocal microscopy to image chromaffin cells labeled with NS521. Chromaffin cells were separated into norepinephrine-enriched and epinephrine-enriched fractions by centrifugation on a Percoll gradient. The norepinephrine-enriched cell population shows strong, punctate fluorescence compared to the epinephrine-enriched cell population, which exhibits only marginal fluorescence. To further validate the selective labeling of norepinephrine-containing vesicles, we labeled fixed cells using an antibody against phenylethanolamine N-methyltransferase (PNMT). The NE-enriched cell population stains brightly with NS521 and weakly with the fluorescent PNMT secondary antibody while the opposite is true for the EP-enriched cell population. Next, we used Total Internal Reflection Fluorescence Microscopy (TIRFM) to image the bottom surface of chromaffin cells loaded with NS521. Clear punctate fluorescence consistent with labeling of individual norepinephrine-containing granules was observed. We conclude that NeuroSensor 521 represents a convenient method to selectively stain norepinephrine and dopamine in neuro-secretory vesicles. Supported by the NSF (CHE-1112194) and the NIH (R43 MH096650).

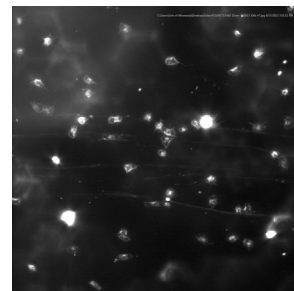
#### 2661-Pos Board B353

##### Darkfield Imaging of Platelet Cytoskeleton-Granule Interactions

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Platelets play a major role in clot formation during hemostasis; however, one of their primary roles, activation, is still poorly understood. When circulating platelets encounter a breach in a vessel wall or certain signaling molecules, such as thrombin or calcium, they begin to activate. Activation is a multivariate processes including the release of granular contents and a rearrangement of the cytoskeleton. To obtain a better fundamental understanding of platelet activation, the interplay between these two processes should be investigated. Live platelets are difficult to image due to their small size and tendency to activate when disrupted (i.e. by attempting to permeabilize the membrane to allow fluorescent molecule access). Fluorescence imaging is further complicated by the fact that platelets do not contain a nucleus, preventing transfection with plasmids that encode fluorescently tagged proteins. This work overcomes these challenges by using darkfield microscopy to image platelets during activation. Darkfield microscopy results in bright, defined images of platelets without exogenous labels. In addition, platelet granules can be tracked using darkfield. Finally, platelet membrane components can be fluorescently labeled and combined with the darkfield imaging for co-localization studies.



#### 2662-Pos Board B354

##### Direct Measurement of Secretory Vesicle-Plasma Membrane Tethering Interactions by Correlated AFM Force-Clamp and TIRF Microscopy

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The molecular mechanisms behind the tethering of secretory vesicles to the plasma membrane are not well understood. To study these mechanisms, we use a combined AFM/TIRF method applying forces to GFP-tagged secretory granules tethered to PC-12 cell membrane sheets. In the experiment shown in the figure, the AFM tip captured a granule, and was then lowered onto the sheet (a-b). Pulling forces were applied, ranging from ~100pN to ~700pN (c-e). Extension and rupture events are apparent as transient variations in the